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Measuring *N*-acetylaspartate synthesis *in vivo* using proton magnetic resonance spectroscopy

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ABSTRACT

N-Acetylaspartate (NAA) is an important marker of neuronal function and viability that can be measured using magnetic resonance spectroscopy (MRS). In this paper, we proposed a method to measure NAA synthesis using proton MRS with infusion of uniformly ^{13}C-labeled glucose, and demonstrated its feasibility in an *in vivo* study of the rat brain. The rate of ^{13}C-label incorporation into the acetyl group of NAA was measured using a localized, long echo-time proton MRS method. Signals from the ^{13}C satellites of the main NAA methyl protons at 2.02 ppm were continuously monitored for 10 h. Quantification of the data based on a linear kinetic model showed that NAA synthesis rate in isoflurane-anesthetized rats was 0.19 ± 0.02 μmol/g/h (mean ± standard deviation, $n = 12$).

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1. Introduction

N-Acetylaspartate (NAA) is found in high concentration (~10 μmol/g) exclusively in the nervous system (Tallan, 1957; Miyake et al., 1981; Koller et al., 1984; Pan and Takahashi, 2005). It is primarily synthesized from acetyl coenzyme A (acetyl-CoA) and aspartate by NAA synthase (L-aspartate *N*-acetyltransferase; ANAT, EC 2.3.1.17) in neuronal mitochondria, or via cleaving *N*-acetylaspartylglutamate (NAAG) catalyzed by *N*-acetylated-α-linked-amino dipeptidase (NAALADase) along with glutamate, and subsequently exported to the cytoplasm (Patel and Clark, 1979; Bzdega et al., 1997; Luthi-Carter et al., 1998). The major NAA catabolic enzyme aspartoacylase (*N*-acetylaspartate amidohydrolase; EC 3.5.1.15) is located in oligodendrocytes (Kaul et al., 1991; Baslow et al., 1999). NAA has been widely used as a neuronal marker in the study of a variety of cerebral disorders using *in vivo* proton magnetic resonance spectroscopy (MRS) (see Moffett et al., 2007 for a recent review). Abnormalities in NAA synthesis, transport and/or breakdown may contribute to abnormal steady-state NAA concentrations observable in proton MRS spectra of the brain (Clark, 1998; Moreno et al., 2001). Canavan disease, for example, is an NAA metabolic disorder due to *N*-aspartoacylase deficiency which results in elevation of the NAA signal in proton MRS spectra (Kvittingen et al., 1986; Matalon et al., 1988; Burns et al., 1992; Moreno et al., 2001). In contrast to the large body of literature on the total concentration of NAA in various brain disorders, the characterization of NAA synthesis remains scarce. Because NAA synthesis is dependent on mitochondrial metabolism and glucose is the major energy source of the brain under most conditions, ^{14}C and ^{13}C-labeled glucoses have been used as primary substrates to determine the rate of NAA synthesis (V_{NAA}) (Fig. 1). Using an *in vitro* enzymatic method, the activity of ANAT in adult rat brain homogenates was determined to be 0.19–0.58 μmol/g/h depending on specific brain anatomy. The cortex acitivity was found to be 0.29 μmol/g/h by Truckenmiller et al. (1985). The activity of ANAT in the forebrain homogenate of 35-day-old rats was determined to be 0.72 μmol/g/h (Burri et al., 1991). The *in vivo* cerebral V_{NAA} has been directly measured using ^{13}C MRS in both α-chloralose-anesthetized adult rats ($V_{NAA} = 0.7 \pm 0.1$ μmol/g/h) (Choi and Gruetter, 2004) and humans ($V_{NAA} = 0.55 \pm 0.23$ μmol/g/h) (Moreno et al., 2001).

The direct ^{13}C methods are limited by the inherently low sensitivity of ^{13}C MRS. The *N*-acetyl methyl group of NAA is the major resonance in water-suppressed proton MRS spectra. The three magnetically equivalent hydrogen atoms of the methyl group resonate in proton MRS spectra with a single, sharp peak at 2.02 ppm, and therefore NAA has been generally recognized as one of the most reliable markers in brain MRS studies (Fan et al., 1986; Luyten and den Hollander, 1986; Barany et al., 1987). Because of the prominence of the NAA proton signal in MRS and its significantly higher sensitivity compared to ^{13}C-labeled metabolite signals, a potentially useful approach is to determine NAA synthesis by measuring the ^{13}C satellite signals of NAA in the proton MRS spectra. The resonances of the ^{13}C satellite peaks are symmetrically located with respect to the

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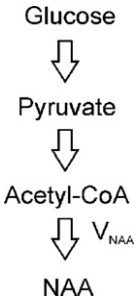

Fig. 1. Schematic representation of synthesis of NAA from glucose. V_{NAA}, NAA synthesis rate.

central resonance of the methyl protons attached to ^{12}C. The intensity ratio of the satellite peaks to the total NAA signal reveals the ^{13}C isotopic enrichment of the acetyl moiety of NAA. Unlike direct ^{13}C methods which require a radio frequency (RF) coil tuned to ^{13}C nuclei and a second RF channel as well as broadband amplifiers, proton MRS is in principle available on all clinical scanners, and by far the most widely used MRS technique. In this study, we demonstrate that V_{NAA} can be measured *in vivo* using a localized long echo-time (TE) proton MRS method with infusion of uniformly ^{13}C-labeled glucose ([U-^{13}C]glucose) to label the *N*-acetyl methyl group of NAA without using a ^{13}C channel. A related but different approach using short echo-time proton MRS for measuring the ^{13}C labeling of glutamate was previously reported (Boumezbeur et al., 2004).

2. Materials and methods

Male young adult Sprague-Dawley rats (169–217 g, $n = 12$) were fasted overnight (>12 h) with free access to drinking water. For preparation, the rats were anaesthetized with isoflurane (1.5%) in a mixture of 70% N_2O/30% O_2. The left femoral artery was cannulated for periodically sampling of arterial blood to monitor blood gases (pO_2, pCO_2), pH, and glucose concentration using a blood analyzer (Bayer Rapidlab 860, East Walpole, MA), and for monitoring arterial blood pressure levels. The left femoral vein was also cannulated for intravenous infusion of [U-^{13}C]glucose (99% enrichment, Cambridge Isotope Labs, Andover, MA, 20% w/v). The glucose infusion protocol consisted of an initial bolus of 162 mg/kg/min of 1.1 M [U-^{13}C]glucose in the first 10 min followed by an approximately constant infusion rate of 62.8 mg/kg/min. Plasma glucose level was maintained at 19.6 ± 1.6 mM during the experiment. Arterial blood pO_2, pCO_2, mean blood pressure, and pH were maintained at approximately 129 ± 16 mmHg, 36 ± 4 mmHg, 100–145 mmHg, and 7.34 ± 0.04, respectively. All procedures were approved by the National Institute of Mental Health Animal Care and Use Committee.

The *in vivo* MRS experiments were performed on a Bruker 11.7 Tesla spectrometer interfaced to an 89 mm inner-diameter vertical bore magnet. A 15-mm inner-diameter proton surface coil (Li and Shen, 2005) was used for excitation and detection, and was positioned ~0–1 mm posterior to bregma. Adjustment of all first- and second-order shims was accomplished using a fully automatic procedure described previously (Chen et al., 2004) which was based on a fast automatic shimming technique by mapping along projections method (Gruetter, 1993). The single-shot adiabatic 3D localization pulse sequence (Slotboom and Bovee, 1995) was similar to that used in our previously study (Xu et al., 2005) but with a lengthened echo-time (TE = 100 ms). Specifically, the proton MRS pulse sequence used slice-selective adiabatic refocusing with two hyperbolic secant pulses per dimension (Conolly et al., 1991) (2 ms sech pulse, $\mu = 5$, 1% truncation). Chemical shift selective (CHESS) water suppression (Gueron et al., 1991) was used together with outer volume suppression using nominal 90° hyperbolic secant pulses along the x (10-mm slab), $-x$ (10-mm slab), y (3-mm slab), $-y$ (5-mm slab), z (10-mm slab), and $-z$ (10-mm slab) directions. The CHESS and the outer volume suppression pulses were repeated three times. Orthogonal gradient pairs or triplets carefully adjusted to minimize the resultant B_0 shifts were used as crushers for CHESS, outer volume suppression, and for the slice-selective refocusing adiabatic pulses. Proton MRS spectra were acquired from a 6 mm × 3 mm × 6 mm voxel centered on the midline of the brain with 4096 data points, a spectral width of 4000 Hz, and a repetition time (TR) of 3.2 s. For each data block, 440 acquisitions were accumulated over 24 min. After acquisition of each data block, a 6-min interval was used for re-shimming to maintain B_0 homogeneity over a total experimental duration of 10 h. Satellite NAA methyl peaks were analyzed using the MATLAB curve-fitting toolbox (The MathWorks, Inc., Natick, MA). Since NAAG cannot be reliably quantified, the total NAA signal (=NAA + NAAG) was used instead. To minimize interference from the glutamate and glutamine H3 methylene group at 2.09–2.14 ppm regions, the upfield NAA satellite peak at 1.89 ppm was used in the quantification of ^{13}C-labeled NAA with its intensity multiplied by a factor of 2.

3. Results

Fig. 2 shows examples of the localized (6 mm × 3 mm × 6 mm) proton MRS spectra of a normal rat brain at short echo-time (Fig. 2a, TE = 15 ms) and at long echo-time (Fig. 2b, TE = 100 ms) without infusion of [U-^{13}C]glucose. The accumulated FID of each data block was zero-filled to 16 K. Resolution-enhancing Lorentz–Gauss transformation (exponential broadening factor (lb) = −4 Hz, Gaussian broadening factor (gb) = 0.3) was applied before Fourier transform. The spectra were phased using zero-order phase only, without any baseline corrections. These *in vivo* spectra show high sensitivity and

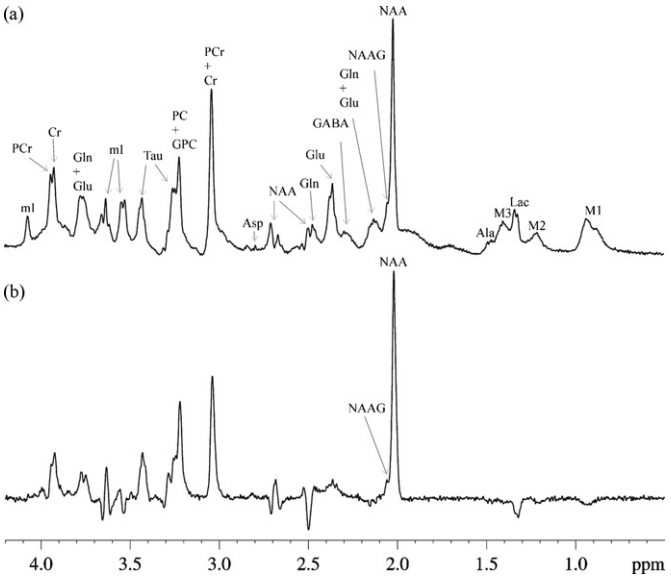

Fig. 2. The localized (6 mm × 3 mm × 6 mm) proton MRS spectra acquired from an individual rat brain with (a) short TE (TE = 15 ms, NA = 440, TR = 3.2 s, lb = −4 Hz, gb = 0.3) and (b) long TE (TE = 100 ms, NA = 1760, all other parameters were the same as in (a)). The spectra were phased using zero-order phase only without any baseline corrections, and were plotted using the same absolute intensity scale. Ala: alanine, Asp: aspartate, Cr: creatine, GABA: γ-aminobutyric acid, Gln: glutamine, Glu: glutamate, GPC: glycerophosphorylcholine, Lac: lactate, mI: myo-Inositol, NAA: *N*-acetylaspartate, NAAG: *N*-acetylaspartylglutamate, PC: phosphocholine, PCr: phosphocreatine, Tau: taurine, M1: macromolecule at 0.92 ppm, M2: macromolecule at 1.21 ppm, M3: macromolecule at 1.39 ppm.